Composition of Granules in Evaporated Milks Stored at Low Temperatures

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Abstract

Compositional analysis of granules from evaporated milks stored at low temperatures (2-4 C) for several years showed them to consist of about 50% phosphate and 21% calcium. The molar ratio of calcium to phosphate is approximately 1:1, which corresponds to the formula $CaHPO_4$. By contrast, granules obtained from evaporated milks stored at room temperatures are known to have a composition corresponding to tricalcium citrate. The granules of evaporated milks stored at low temperatures also contain an insoluble matrix of peptide-like material. After the dibasic calcium phosphate was dissolved with M HCl, this material remained. A part of this material can be dissolved in NaOH, after which it is soluble at neutrality. Amino acid analysis of this base-soluble fraction shows it to be high in glutamic acid.

When evaporated milks are stored for considerable periods of time at room temperature, a white granular material forms on the container walls; these granules, when dislodged by agitation, settle to the bottom of the can. Earlier studies have shown this material to consist primarily of calcium citrate. These particles, when present, are considered a defect in the product.

In this paper, we report the development of grossly similar granules in evaporated milks stored at low temperatures and demonstrate that, though similar in appearance to the material formed at room temperature, these granules differ markedly in composition. We also suggest that the mechanism responsible for their formation varies with the storage temperature.

Materials and Methods[1]

The evaporated milks stored at room temperature were commercial products between eight months and 3 yr old. The evaporated milks stored at 2-4 C were commercial products 7 yr

[1] Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

old. Granular material had been observed in these milks when they were 3 yr old.

The granular material was collected and prepared for analysis as follows: The milks were poured on to a 60-mesh screen and the granules washed free of occluded milk with tap water. They were then thoroughly washed with distilled water, dried with acetone, and held under vacuum overnight to remove the last traces of surface moisture.

Infrared (IR) spectra were obtained with a Perkin-Elmer Model 421 instrument. Solid samples at a concentration of 2% were analyzed by a micro KBr pressed pellet technique.

Nitrogen was determined by a micro-Kjeldahl procedure (1).

The granules were ashed in a muffle furnace at 550 C for 16 hr. Calcium was determined on the ash by the back-titration method of Yalman et al. (10).

To determine phosphorus, it was necessary to add 1.5 parts calcium chloride to 1 part granules prior to ashing. The mixture was dissolved in M HCl, evaporated to dryness, and ashed as above. Phosphorus was then determined by formation of phosphomolybdic acid, followed by reduction with 1-amino-2-naphthol-4-sulfonic acid (6).

Organic material present in the granules was isolated as follows: Granules in the ratio of 1 g per 20 ml M HCl were stirred until they were well-suspended in the acid. An equal volume of distilled water was added and the suspension was then centrifuged for 2 min in a clinical-type centrifuge. The sediment was washed twice by resuspending it in an equal volume of M HCl and recentrifuging. The washings were discarded. The sediment was washed from the centrifuge tube and was made basic by the drop-by-drop addition of M NaOH until the suspended material dissolved. Part of the suspended material was insoluble at any pH and was removed by centrifuging. The remaining solution was desalted by passing it over the G-10 Sephadex column with distilled water as the eluting solvent. After lyophilizing, 17 mg of dry product were obtained per gram of granules. This material was then analyzed for nitrogen and amino acid content. It gave a negative Molisch test.

Milk diffusate was collected either by dialyzing 250 ml distilled water vs. 40 liters of fresh skimmilk for three days at 4 C or by negative-pressure dialysis.

All reagents were analytical grade except the chloroform and KBr used in making IR pellets; these were spectroscopic grade.

Results

Figure 1 is a photomicrograph of granules from evaporated milks stored at room temperature for 10 months. It has been reported that particles of this type range in dimension from the microscopic to 8 mm in diameter. Particles in this figure are approximately 1.3 mm in diameter.

A photomicrograph of the granular material isolated from 7-yr-old evaporated milks stored at 2-4 C is shown in Figure 2. It can be seen that, though smaller in diameter, these granules appear grossly similar to those shown in Figure 1.

The IR absorption spectra of granules isolated from evaporated milk stored at room tem-perature and of a sample of pure calcium citrate are shown in Figures 3 and 4. The agreement in the location of the major absorption bands in the two spectra is excellent. Differences in band intensity can be accounted for by slight variations in the concentration of sample in the KBr pellets used for the analysis.

Table 1 shows the chemical analysis of the granules from milks stored at 2-4 C. The composition of the granular material can mostly be accounted for by calcium, phosphorus, and water. However, the amount of nitrogen found cannot be considered insignificant. Amino acid analysis of the base-soluble organic fraction

TABLE 1

Composition of granules from evaporated milks stored at 2-4 C

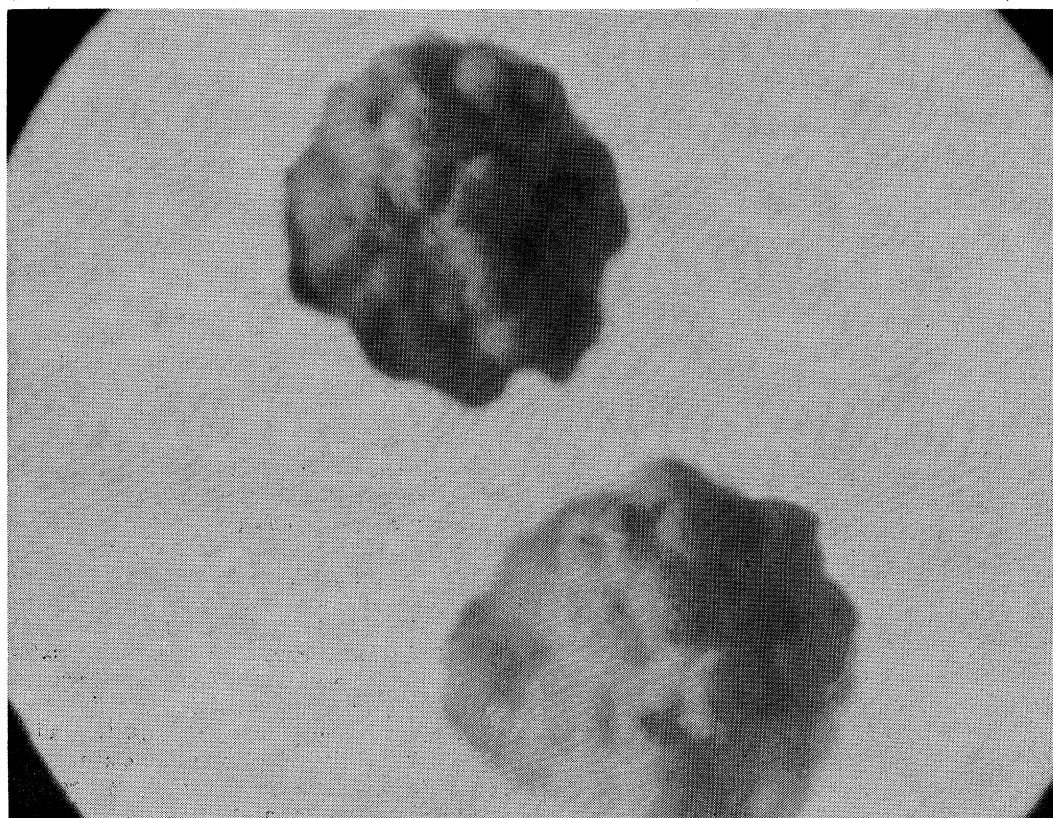| Component	Per cent
Calcium	20.9
Phosphate (as PO_4)	50.0
Nitrogen	.9
Ash	67.9
Water	17.6

FIG. 1. Photomicrograph of granules isolated from evaporated milk stored at 21 C for ten months. Film: Kodak Plus-X Pan. Microscope magnification: 17.5 ×.

FIG. 2. Photomicrograph of granules isolated from evaporated milk stored at 2-4 C for 7 yr. Film: Kodak Plus-X Pan. Microscope magnification: 17.5 ×.
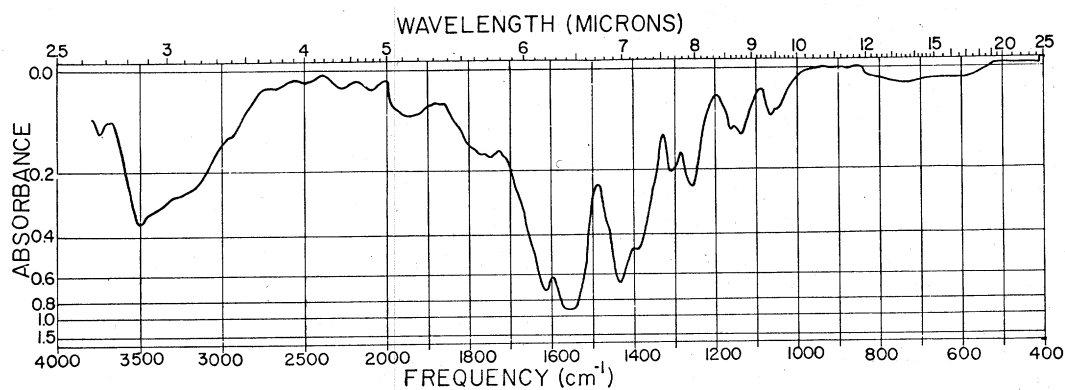

FIG. 3. Infrared absorption spectrum of granules isolated from evaporated milk stored at 21 C for ten months.

showed it to contain practically all of the amino acids found in a typical protein, being only slightly high in glutamic acid.

As shown in Figure 5, the IR absorption spectrum of the granules from evaporated milks stored at 2-4 C is markedly different from the spectrum of calcium citrate. There is no agreement in the location of the major absorption bands.
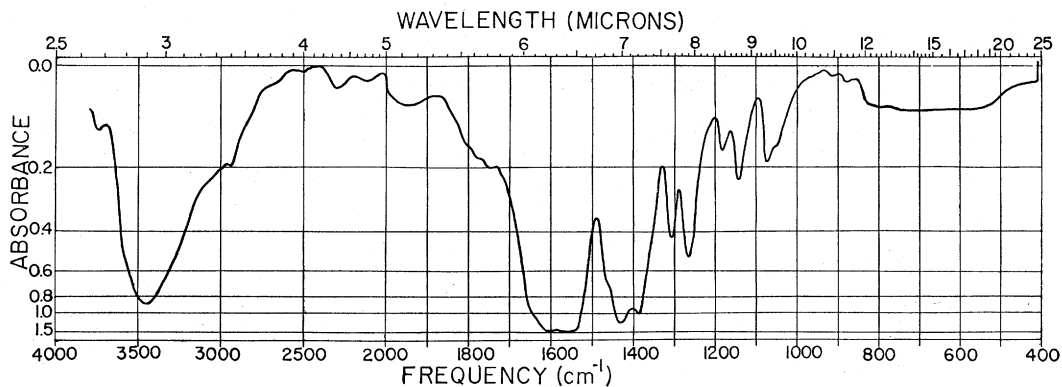
Figure 6 shows the IR spectrum of the de-

WAVELENGTH (MICRONS)

FIG. 4. Infrared absorption spectrum of pure tricalcium citrate.
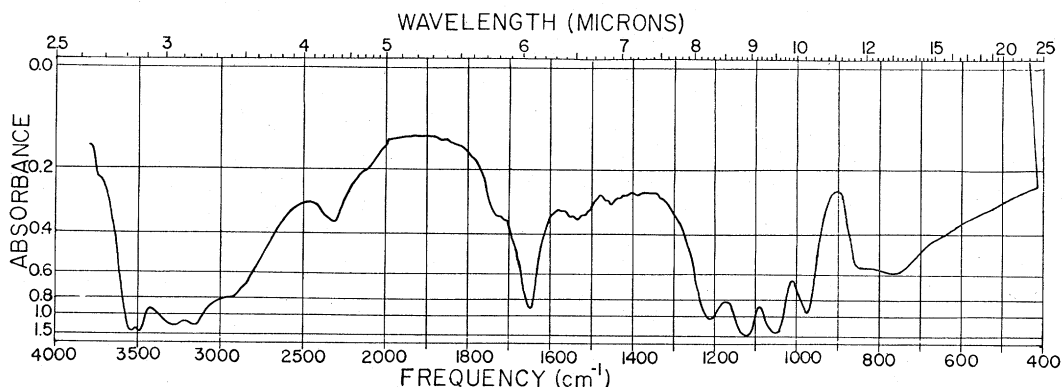
WAVELENGTH (MICRONS)

FIG. 5. Infrared absorption spectrum of granules isolated from evaporated milk stored at 2-4 C for 7 yr.
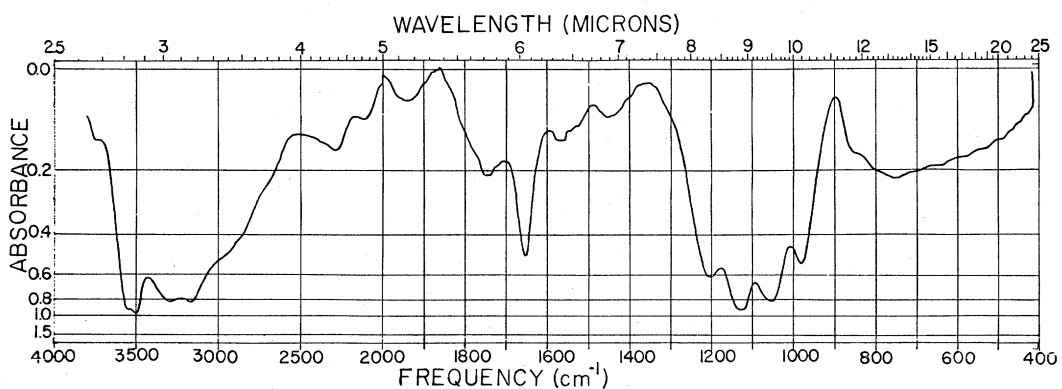
WAVELENGTH (MICRONS)

FIG. 6. Infrared absorption spectrum of deposits from milk dialysate stored at 4 C for one month.

posits found in flasks of milk dialysate stored at 4 C for 1 month. It can be seen that this spectrum is very similar to that of Figure 5, in regard to the location of the major absorption bands and their relative intensities.

Discussion

Gould and Leininger (7) and Deysher and Webb (4, 5) almost simultaneously studied the composition of the granular deposits in stored evaporated milks and reported them to be primarily composed of calcium citrate. These analyses were based on the conversion of citric acid to pentabromoacetone. Although the method, as they used it, could not be considered completely specific, we have confirmed their work

by IR absorption spectroscopy, as shown in Figures 3 and 4.

In view of the good agreement in these spectra, it was surprising to find that the IR spectrum of granules from evaporated milks stored in the cold for extended periods, as shown in Figure 5, differed markedly in appearance from those of the preceding figures.

Compositional analysis of these granules showed them to consist of about 50% phosphate and 21% calcium. The molar ratio of calcium to phosphate is approximately 1:1, which corresponds to the formula $CaHPO_4$. In addition, these granules also contain an insoluble matrix of peptide-like material. To date, all attempts to fully solubilize this material have failed. The material could neither be directly leached from the particles by the solvents used nor could the residue be completely dissolved in base after dissolving away the inorganic constituents with weak acid. Amino acid analysis of the base-soluble fraction showed that it contained a slightly elevated amount of glutamic acid.

Better insight into the nature of the granules formed at low temperatures was obtained from an examination of the deposits formed from milk dialysate stored in the cold. When a small volume of water is dialyzed to equilibrium against a large volume of milk, a solution is obtained which, theoretically, should contain all low molecular weight constituents found free in milk at their true concentrations. When these dialysates are stored at 4 C for several weeks, granular deposits form and settle out. As shown in Figure 6, the IR spectrum of this material is quite similar to the spectrum of the granules formed in evaporated milks during cold storage, as shown in Figure 5.

From a consideration of the source of these two figures, it can be inferred that the granules arise from a combination of hydrated dibasic calcium phosphate and nitrogenous organic material of molecular weight below 10,000.

The strong absorption peak at 1,640 cm^{-1}, as seen in Figures 5 and 6, can be found as a less intense band in the spectra of many inorganic hydrates (3). Lowering the water content of the granules by heating under vacuum reduced this peak only slightly. Apparently, the absorption observed in this region of the IR spectrum of the granules arises primarily from NH deformation vibrations and C = O stretch of the organic material present.

Lecomte et al. (8) have studied the effect of hydration on the IR spectrum of pure dibasic calcium phosphate. They have demonstrated the presence of two kinds of water in the hydrated calcium phosphate, as shown by four

bands in the IR spectrum, forming two doublets whose components have approximately equal intensity. These bands are found at 3,158-3,268 cm^{-1} and 3,488-3,522 cm^{-1}. The doublet 3,488-3,522 cm^{-1} plus a supplementary band at 663 cm^{-1}, arises from water which is an integral part of the crystalline structure and vibrates with it. The doublet arising at 3,158-3,268 cm^{-1} occurs from water of hydration, which is weakly bound and can be removed by heating under vacuum. The doublets present in this region in Figure 5 and 6 (3,170-3,300 cm^{-1} and 3,500-3,560 cm^{-1}) are all shifted to a slightly higher frequency. This can be accounted for partly by the presence of hydrogen bonds in the molecule and by NH stretch from the nitrogenous material present in the granules. Heating the granules overnight reduced the intensity of the doublets, but caused no change in frequency. No obvious band at 663 cm^{-1} can be observed in the granule spectrum. However, this band would be obscured by the very broad shallow bands in the 500-800 cm^{-1} region believed to be produced by NH$_2$ wag and OH out-of-plane bend.

It is difficult to infer structure from the absorption in the region 970-1,210 cm^{-1}, because the infrared absorption of the phosphate radical is greatly influenced by symmetry and hydration.

The absorption peaks at 970, 1,050, and 1,120 cm^{-1} correspond very closely to the strong bands at 990, 1,050, and 1,125 cm^{-1} found in the spectrum of pure hydrated dibasic calcium phosphate published by Miller and Wilkins (9). Corbridge and Lowe (3) attribute these multiplet bands to some sort of P$^+$-O$^-$ ionic stretching. The peak at 1,210 cm^{-1}, however, is much harder to characterize. This peak is absent in the spectrum of pure dibasic calcium phosphate which, in turn, contains a weak band at 1,350 cm^{-1} which is absent in the spectrum of Figures 5 and 6. In the absence of hydrogen bonding, a moderate peak can be found in the frequency range 1,350-1,175 cm^{-1} in the spectra of phosphates, which is attributed to the P = O stretching vibration, the location of the band depending on the type of phosphate ion. When hydrogen bonding can occur with either OH or NH groups, the P = O frequency is subject to shifts of 50-80 cm^{-1}. In addition, the intensity of the P = O vibration is considerably increased (2). Therefore, it is possible that the peak at 1,210 cm^{-1} arises from strong hydrogen bonds between P = O and OH molecules of water of crystallization and between P = O and the NH groups free in the nitrogenous material found in the low-temperature granules.

If the latter assumption is taken, granules forming in evaporated milks stored at low temperatures may result from precipitation of dibasic calcium phosphate on to which available peptides or free amino acids are bound through hydrogen bonds. The low temperature and possibly the bonded peptides should depress the solubility of the calcium phosphate to the point where granule formation is possible.

Since the solubility of hydrated forms of calcium citrate decreases with increasing temperature, elevated storage temperatures promote its precipitation, apparently without complexing nitrogenous materials. On the other hand, the solubility of the hydrated calcium phosphate is increased with increasing temperature to the point where it remains in solution.

From the data presented here, we have concluded that storage conditions have great influence on the type of granule formed.

Acknowledgment

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